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Repeatability and Reproducibility (RR) of Bioelectric Impedance Vectors in Brazilian Children with Normal Body Mass

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Abstract

Background: Bioelectrical analysis measures two bioelectrical vectors: resistance (R) and reactance (Xc). Resistance is the pure opposition of a biological conductor to the flow of an alternating current through the intra and extra-cellular ionic solution and it is inversely related to the dynamics of body fluids and body composition.

Objective: The purpose of this study was to determine the reference values of the indexes bioelectrical impedance (BI) for children of normal body mass index in southeastern Brazil of middle-income country.

Methods: Two hundred eighty-one children with normal body mass index were included in the study (135 female and 146 male), aged 4 to 129 months, selected from federal public urban school in São Paulo, São Paulo, Brazil, where bioelectrical impedance values resistance (R) and reactance (Xc) values were measured in order to establish reference values of these parameters.

Results: The anthropometric variables, body mass index, z-scores and bioelectrical impedance parameters were evaluated. For both genders, the mean and standard deviation of anthropometric variables were: age (months): 73.42 $\pm$ 34.65; weight (kg): 23.5 $\pm$ 9.46; height (m): 1.16$\pm$0.22; BMI (kg/m^2): 16.65$\pm$1,75; Xc (ohms): 63.92$\pm$9.6; R (ohms): 749$\pm$75.26. For analysis, the children were stratified into three groups for each gender, being divided by ages: 4 to 23 months; 24 to 71 months and 72 to 129 months. Linear regression analysis showed R had a significant progressive decrease with age (p=0.0003) while Xc had a progressive increase (p=0.0065) with age increase. We analyzed by multiple regression the associations between R and Xc with anthropometric variables by age group to establish the reference values, confidence intervals and the tolerance limits for a new individual observation. Test-Retest Repeatability between three repeat and consecutive measurements was considered excellent. Intraclass correlation coefficient and Bland - Altman reproducibility was for reactance 0.755, Resistance 0.98 and phase angle was 0.93.

Conclusion: The BI reference values were established, in a field where there is a relative lack of publications, and we collected relevant information about resistance and reactance in a population of middle income setting that could be used in epidemiologic studies and could be used reference value in children with altered body composition.

Bioelectrical impedance analysis (BIA) is a fast, and inexpensive method that has been widely applied to evaluate the body composition for over thirty years [1,2,3,4].

Impedance (Z) is an electrical property of ionic conduction measurable through soft tissue (except fat and bone) and measures two bioelectrical vectors: resistance (R) and reactance (Xc).

The resistance vector is the opposition of a conductor to the flow of alternating electric current through intracellular and extracellular ionic solutions and represents the real part of the impedance. In a biological conductor, current is mainly carried by ions and aqueous solutions. The reactance vector is the capacitance produced by tissue interfaces and cell membranes and represents the imaginary part of Z (PICCOLI et al, 1998). It is reciprocal to electrical capacitance, that is, it is the voltage stored by a biological capacitor for a brief period of time (LUKASKI et al, 1996).

When an electric current is applied, when encountering a capacitive element, a phase difference is created between the current and the applied voltage, determined by the reactive component of R, which is represented geometrically by the phase angle. (BAUMGARTNER et al, 1988; PICCOLI et al, 1998). The phase angle (in degrees) is the angle that the impedance vector makes with its resistive component and is calculated as the arctangent of the ratio of reactance to resistance.

The use of bioelectrical impedance to estimate body composition variables is based on the hypothesis that fat-free tissues are good conductors at fixed frequencies and poor conductors under alternating current, when compared to adipose tissue.

The body bioelectrical impedance technique is useful in the analysis of body composition, as it allows health professionals to manage and prevent nutritional problems. Additionally, the growing interest in the study of body composition and its variations as a method of assess in nutritional status grows over the years as well as recognition of its importance for the assessment of healthy and sick individuals [7,8].

BI has a hypothetical inverse relationship to the body's volume and can be used in regression prediction models to estimate total body water (TBW). It is based on a bi-compartmental model, which divides the body into lean mass (LM) -high conductivity, a fact that reduces resistance (R) and fat mass (FM) – low conductivity that increases body resistance (R).

Bioimpedance vectors (resistance and reactance) are rarely referred to in the literature as vectors that reflect the dynamics of body fluids and the electrical properties of tissues. How impedance vectors behave in individuals with body composition distortions, such as critically ill patients, is a challenge that has not yet been fully clarified.

However, there has been a growing interest in the use of these body bioimpedance vectors, reactance (Xc) and resistance (R), as a way to assess hydration (resistance) and cell permeability disorders (reactance) [5].

The monitoring of primary indices of bioimpedance, resistance (R) and reactance (Xc) has great applicability in the scenario of critically ill patients, as it can be considered a non-invasive way of predicting the patient's prognosis, acute malnutrition, assessment of fluid responsiveness, cumulative fluid balance considering Xc can reflect the from a biophysical point of view changes in the permeability of the endothelium and cell membrane. [6-10].

There is a relative lack of publications in the field of bioelectrical parameters reference values on specific population such as low- and middle-income countries for this reason, few studies are reported to accurately assess nutritional individual deviations in relation to these population mean and to analyze the role of bioelectrical parameters on various outcomes in the clinical setting and epidemiological studies [11,12].

The purpose of this study was to evaluate the repeatability and reproducibility of BIA in children with normal body mass index in southeastern Brazil.

Methods

Data were collected in healthy children aged 4months to 129 months at a federal elementary school in São Paulo city, Brazil. The children belonged to families that have the socio-economic status of the majority of the Brazilian population, being in the middle-income population of Brazil. The protocol was approved by the committee of ethics on research and the school's authorities.

Study Population

Three hundred, twenty-seven children of both genders were recruited after an interviewing their parents and obtaining a signed written informed consent. The admission criteria for this study were: a) z- score between –2 and +2, b) fasting state > 3 hours and c) no vigorous physical activity in the 24 hours prior to the tests. The exclusion criteria were: a) undernutrition [z-score <-2], b) obesity [z-score $\geq$ +2], c) acutely ill children, and d) those who were under medications.

Anthropometric measurement –

The anthropometric measurements were obtained by the principal investigator who was previously trained to perform the measurements. The anthropometric measurement procedures were undertaken in strict accordance with the methodology described in previously published papers. [8,9,10].

The body weight was measured to a precision of 0.1 Kg with an electronic beam-balance in children over 23.9 months of age. In children under 23.9 months, the body-weight was measured to a precision of 0.01 kg using an electronic scale. The body-height was measured by a stadiometer to a precision of 0.1 cm for all age groups. The children were measured without shoes and wearing underwear. The age, body weight and height were used to calculate the z-score. We used the relationship weight-for-height (W/H index) for the nutritional assessment of the children over 23.9 months of age and for children under 23.9 months, the weight-for-age (W/A index) and weight-for-height (W/H index). The values obtained were compared to standard reference values.

We used version 1.02 of the ANTHRO program from the Nutrition Division of the Disease Control Center (CDC). To calculate the z score, comparisons were made between the z scoresobtained with the curves of the National Center for Health Statistics (NCHS), using cutoff valuesto define the nutritional condition ± 2 z scores. We determined the body mass indices (BMI) -weight (kg) divided by the square of height in meters - for each child, which were also compared with the NCHS values. Thus, only children with a Z score and BMI within the normal values established by the NCHS were included in the main study [13]

Bioimpedance measurements

Whole-body electrical resistance and reactance were measured with a bioimpedance analyser (Biodynamics model 310; Biodynamics Corporation, Seattle, WA) of alternate current at 800 μA and 50 kHz in tetrapolar arrangement.

Whole-body electrical resistance and reactance were measured with a bioelectrical impedance analyzer that measure resistance and reactance independently and separately. (Biodynamics model 310; Biodynamics Corporation, Seattle, WA) of alternate current at 800 μA and 50 kHz in tetrapolar arrangement. Oil was removed from the skin by cleaning it with alcohol. No direct contact was made

with the child's skin during measurements, and the children were calm and relaxed [14,15,16]. For children under 18 months of age (where cooperation was more difficult), we made a cylindrical non-conducting polyethylene, a type of non-toxic, lightweight, flexible, and waterproof plastic with the objective of positioning the children correctly, i.e., in dorsal decubitus with arms and legs separated and in abduction at 30 degrees from the trunk. That frame was not used with older children, and the supine positioning was maintained. We positioned the electrodes in pairs on the right side of the body in the following anatomical positions: 1- Right hand: The current injector electrode was positioned in the middle of the dorsal surfaces of the hand proximal to the third phalangeal-metacarpal joint. The detector electrode was placed 4 cm below the wrist (group 1) or medially between the distal bony prominences of the radius and ulna (group 2 and 3);2- Right foot: the current injector electrode was positioned in the middle of the dorsal surfaces of the foot to the third metatarsal-phalangeal joint. The detector electrode was placed 4 cm on the ankle (group 1) or medially between the medial and lateral malleoli at the ankle (group 2 and 3). Before each test, the master power switch of the analyzer was turned off and on. After pressing the on key, the analyzer performs a self-test to check the internal calibration in accordance with the recommendation of the manufacturer.

Repeatability and reproducibility of measurements

To preliminarily determine the repeatability and reproducibility of measurements, we performed 3 consecutive measurements of R and Xc in all children (n = 280). The equipment was turned off and after pressing the on key waiting for the self-test and reprogramming the data for a new reading of R and Xc. This procedure was performed sequentially 3 times, and, during the reprogramming intervals, without the child's movement. In 97 healthy children age from 72 months to 123 months (n=97) repeatability and reproducibility measurements were collected on two consecutive days, also.

Statistical Analysis

Descriptive analysis was expressed as mean, standard deviation and 95% confidence intervals (CI). The inferential statistical analyses were performed using GCM and REG procedures of the statistical software package SAS (Version6.0). Bivariate correlations and stepwise maximum R2 was performed by multiple linear regression analyses to evaluate the strength and variability of R and Xc with weight(W), height (H) by age and gender. A p value <0.05 was considered statistically significant. [18].

Multiple regression models and Pearson's correlation coefficient were used to assess the strength and relationship between R and Xc with weight (W), height (H), age and sex. The fitted models were different from each other, according to the sex and age group. Multiple regression models were then fitted for R and Xc as functions of weight and height for each sex, considering age groups adapted from the Committee on Nutrition Advisory to CDC and Waterloo et al. [10,17]. Residual analysis was developed to evaluate the adequacy of the fitted models. The fitted regression models, for each sex and age group, according to the models:

$R = a0 + a1*H + a2*W + \varepsilon$ and $Xc = b0 + b1*H + b2*W + \varepsilon$

They were used to predict the average R, average Xc and confidence intervals. The statistical analysis was accomplished by the SAS system V 6.0(SAS) Institute Inc, 1989. Repeatability and reproducibility were analyzed using IBM SPSS version 20.0 for Windows (IBM Corp. Armonk, NY, USA) and EXCEL for Windows version 10.0. Repeatability refers to the variation in repeated measurements made on the same subject by the same operator under identical conditions [19,20]. Reproducibility is the additional variability introduced when measurements are made on same subjects but under different days. In the study was considered two consecutive days.

Repeatability and reproducibility studies can be assessed using reliability (inherent variability in the true difference between measurements) and /or agreement (the quantified variation between measurements). The analysis of reliability in this study was determined using intraclass correlation coefficient (ICC) and agreement using Bland-Altman analysis. ICC values ≤ 0.5, 0.5-0.75, 0.75-0.9 and ≥ 0.9, were indicative of poor, moderate, good and excellent reliability. [19,20]

Results

The variables were collected in 327 children during the same period of year. Children excluded were: six were undernourished, fourteen obese and twenty-six had other exclusion criteria. The final study population consisted of 281 healthy children with normal body mass index. Children were previously stratified by three age-group due to low number: a) 4 months to 23 months (group 1), b)24 months to 71 months (group 2), and c) 72 to129 months (group 3). The subject characteristics are presented in Table 1.

Table 1: Demographic characteristics of all children.

Group		Age (months)		Height (cm)		Weight (Kg)		BMI		R (Ohm)		Xc (Ohm)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	Both gender*	10.3	4.6	71.3	6.3	8.7	1.9	8.71	1.85	801	96	51	8
2	M (n=39)	51.7	13.6	106.6	10.9	18.4	4.0	16.02	1.35	750	64	63	9
	F (n=37)	56	13.1	106.5	9.2	18.4	3.1	16.10	1.17	765	64	65	8
3	M (n=94)	97	15.8	130.3	9.2	28.8	6.4	16.79	1.86	720	60	67	8
	F (n=73)	98.8	15.8	131.5	10.6	29.8	7.8	16.92	2.12	750	75	67	8
Total	M (n=146)	76.48	31.96	1.19	0.20	24.35	8.45	16.67	1.72	729.53	62.98	64.21	9.28
	F (n=135)	70.13	37.18	1.12	0.28	23.95	14.79	16.63	1.80	770.56	81.78	63.61	9.98
Total	n=281	73.42	34.65	1.16	0.23	23.5	9.46	16.65	1.75	749	75.26	63.92	9.6

Group 1 = 4 months < age < 23 months; Group 2 = 24 months < age < 71 months; Group 3 = 72 months < age < 123 months. Height, cm; Weight, Kg; R, resistance in ohm (Ω); Xc, reactance in ohm (Ω); SD, standard deviation.

Linear regression analysis was performed to evaluate if age-group stratification was appropriate to study the variability of the resistance and reactance in relation to anthropometric variables. Figs. 1 and 2 shows that the stratification was appropriate. In both graphics there are two inflection points, the first point at 23 months and the second at 71 months. These two points were interpreted as indicative of the resistance and reactance variations imposed by growth and development. The straight lines were significantly different for the resistance (p=0.0003) and reactance (p=0.0065).

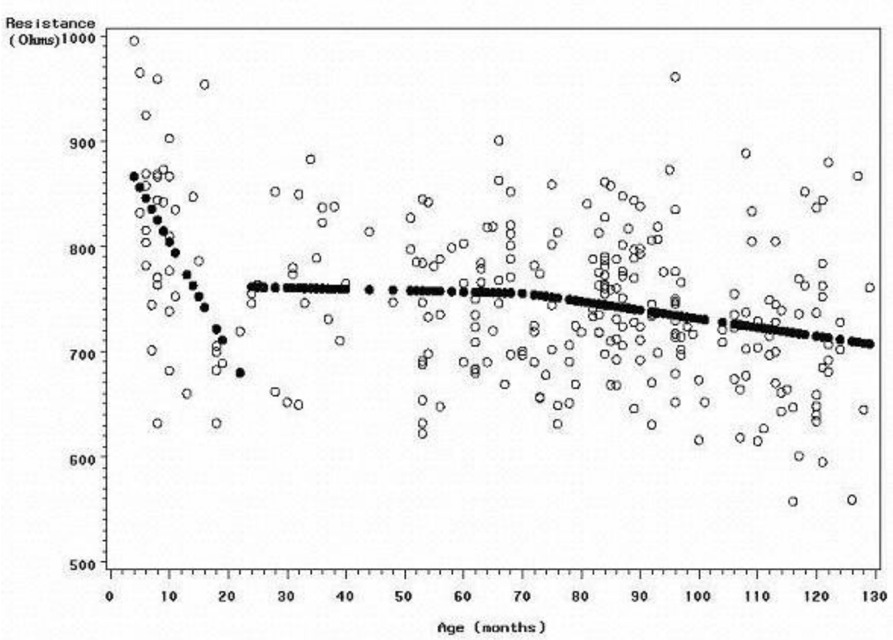

Figure 1: Relationship between measured (white) and predicted (black) resistance values according to age. The regression line predicted for the three age groups studied were significantly different (p =0.003).

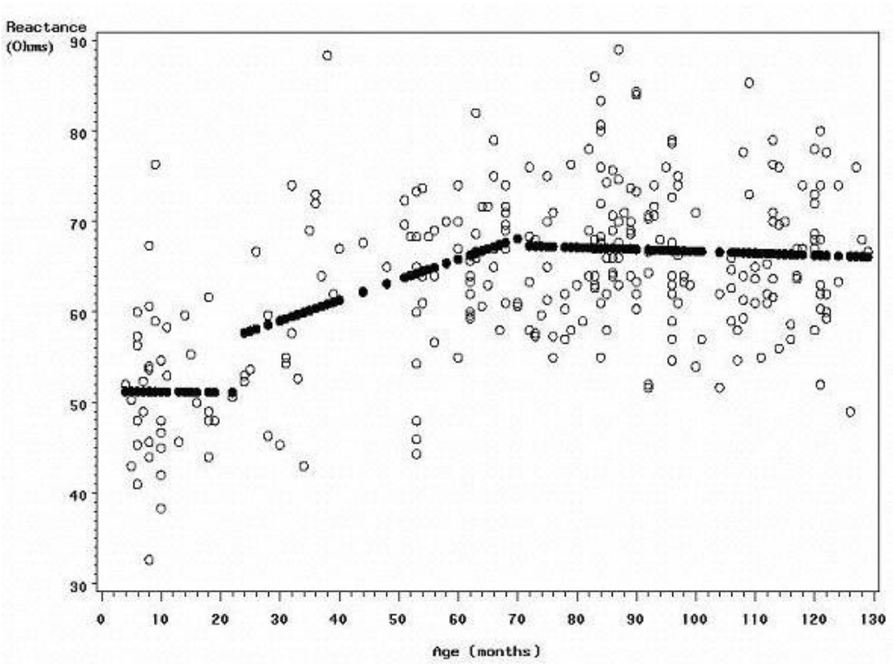

Figure 2: Relationship between measured (white) and predicted (black) reactance values according to age. The regression line predicted for the three age groups studied were significantly different (p = 0.0065).

Correlation between Bioelectrical Impedance Components and Anthropometric Variables Multivariate regression models were used to analyze the correlations between resistance and reactance with anthropometric variables. The purpose of these models was to establish confidence intervals for R and Xc for normal children and tolerance intervals for a new observation. Tables 2 and 3 lists the multivariable regression equations.

Table 2: Prediction of the resistance according to age, body weight, body height for three study-groups by age and genders.

Group/sex		N	a_0	a_1	a_2	r^2	SEE	p
G1	Both	38	600.44^a	10.86^{ns}	-65.89^c	0.41	75.39	0.0001
G 2	Male	39	636.82^d	3.22^{ns}	-12.63^e	1.14	60.75	0.07
	Female	37	608.83^d	4.00^{ns}	-14.73^{ns}	0.11	62.71	0.14
G 3	Male	94	467.48^d	3.96^c	-9.14^d	0.29	51.37	0.0001
	Female	73	268.46^f	6.50^d	-12.54^d	0.39	59.43	0.0001
$R=a_0+a_1*H+a_2*W$; R = resistance (ohm); H= Height (cm); W = weight (Kg); Group 1 = 4 months < age < 23 months; Group 2 = 24 months < age < 71 months; Group 3 = 72 months < age < 123 months. $^a p<0.02$; $^c p<0.002$; $^d p<0.0001$; $^e p<0.05$; $^f p<0.006$. NS = non- significant.								

Table 3: Prediction of reactance according to age, body weight, body height for three age study-groups and gender

Group	Gender	N	b_0	b_1	b_2	r^2	SEE	p
1	Both	38	62.92^a	-0.24^{ns}	0.60^{ns}	0.008	7.43	0.87
2	Male	39	27.17^{ns}	0.52^{ns}	-1.16^{ns}	0.085	8.21	0.21
	Female	37	13.15^a	0.71^a	-1.27^{ns}	0.204	6.87	0.02
3	Male	94	50.73^a	0.21^{ns}	-0.42^{ns}	0.031	7.91	0.23
	Female	73	44.12^a	0.33^{ns}	-0.73^a	0.147	7.16	0.0038
$Xc= b_0+b_1*H+b_2*W$; Xc = reactance (ohm); H= Height (cm); W = weight (Kg); Group 1 = 4 months < age < 23 months; Group 2 = 24 months < age < 71 months; Group 3 = 72 months < age < 123 months. $^a p<0.02$; NS = non- significant.								

Due to the small number of children for each gender in age group 1, one model was adjusted for both genders. Pearson's correlation coefficient between anthropometric variables and bioimpedance vector components are described in Table 4.

Table 4: Correlation of resistance and reactance with body weight, body height for age study-groups and gender.

	Group 1	Group 2		Group 3	
	Both genders	Male	Female	Male	Female
	Resistance (Ohm)				
Height (cm)	-0.48^a	-0.22^b	-0.07^b	-0.22^b	-0.24^c
Weight (Kg)	-0.60^a	-0.30^e	-0.20^b	-0.44^a	-0.44^a
	Reactance (Ohm)				
Height (cm)	-0.07^b	0.08^b	0.46^d	-0.04^b	-0.04^b
Weight (Kg)	-0.05^b	-0.00^b	0.32^c	-0.12^b	-0.12^b
$^a p<0.001$; ^{b} p= NS; $^c p<0.05$; $^d p<0.005$; $^e p<0.06$.); Group 1 = 4 months < age < 23 months; Group 2 = 24 months < age < 71 months; Group 3 = 72 months < age < 123 months.					

Weight and height were negatively correlated with resistance in all age groups. The reactance was positively correlated with weight and height in females in all age groups. Boys and girls did not differ in age, body weight and body height but girls had a higher resistance than boys in groups 2 and 3. This difference in body resistance between boys and girls was not found in the infants (group 1). Reactance increases with age, having few variations between genders (Table 5).

Table 5: Estimates and 95% tolerance intervals for resistance and reactance for three age-study groups.

Group	Gender	Mean	Lower 95% TL	Upper 95% TL
	Resistance (Ohm)			
1	Both	880	707	1053
2	Female	765	744	787
	Male	748	728	769
3	Female	749	732	767
	Male	721	708	733
	Reactance (Ohm)			
1	Both	51	48	54
2	Female	65	63	68
	Male	63	60	66
3	Female	67	65	69
	Male	67	65	68

Group 1 = 4 months < age < 23 months; Group 2 = 24 months < age < 71 months; Group 3 = 72 months < age < 123 months.
TL= tolerance limits; Lower 95% TL= lower limit; Upper 95% TL= TL upper limit.
Mean estimated value was calculated using the regression models presented in Tables 2 and 3. Tolerance limit for the estimated mean were calculated with the expression:

$$\hat{\bar{y}}_0 \pm zs\sqrt{x_0^T(X^TX)^{-1}x_0}$$

Where x_0 = (W, H) in the estimated regression equation and X the model matrix. Z is the corresponding normal distribution percentile and s the standard error estimate.

The regression models were used to estimate Rand Xc mean and 90% to 99% confidence intervals (CI) for age group and gender. In addition, we used the regression models to estimate the values expected of the impedance vectors and the tolerance limits 90% to 99% for anew observation.

For analysis of repeatability and reproducibility, 97 children were studied belonging to the group between 72 to 129 months (group 3). Measurements were taken under ideal conditions of collaboration according to the technique previously described. The analyzes obtained on days 1 and 2 were carried out under good conditions of the environment and temperature.

Test-Retest Repeatability between three repeat and consecutive measurements was considered excellent. For Reactance test-retest was 0,949. For resistance for test re-test was 0,99. For phase angle test-retest repeatability was 0.98.

Intraclass correlation coefficient and Bland -Altman reproducibility between day 1 and Day 2 for reactance was 0.755. Resistance between day 1 and day 2 was 0.98 and phase angle between day 1 and day 2 was 0.93. The Bland-Altman mean of differences plots are show in figures 3-6.

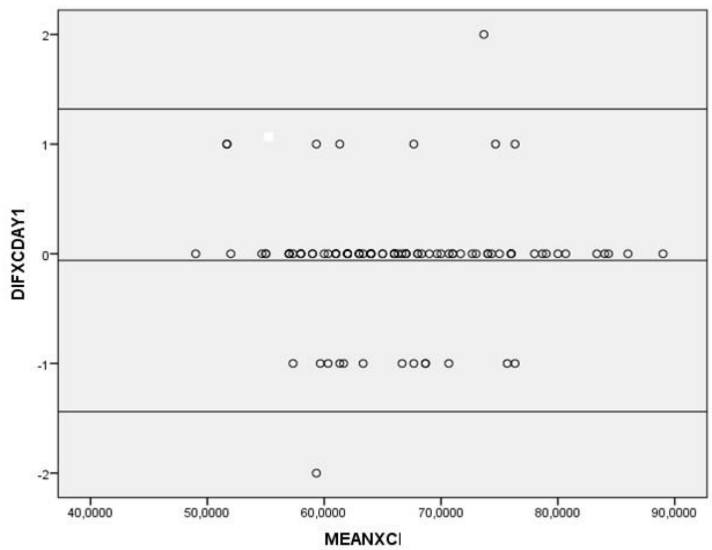

Figure 3: Bland-Altman plots of differences of Reactance against mean of Reactance.

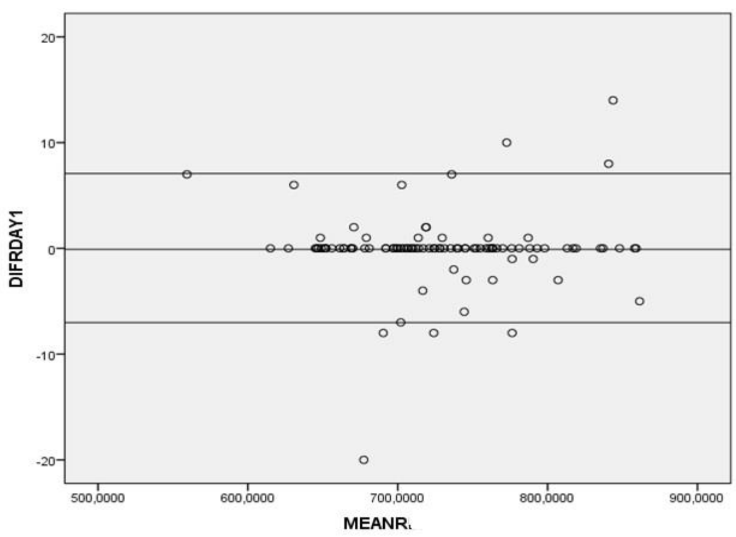

Figure 4: Bland-Altman plots of differences of Resistance against mean of Resistance.

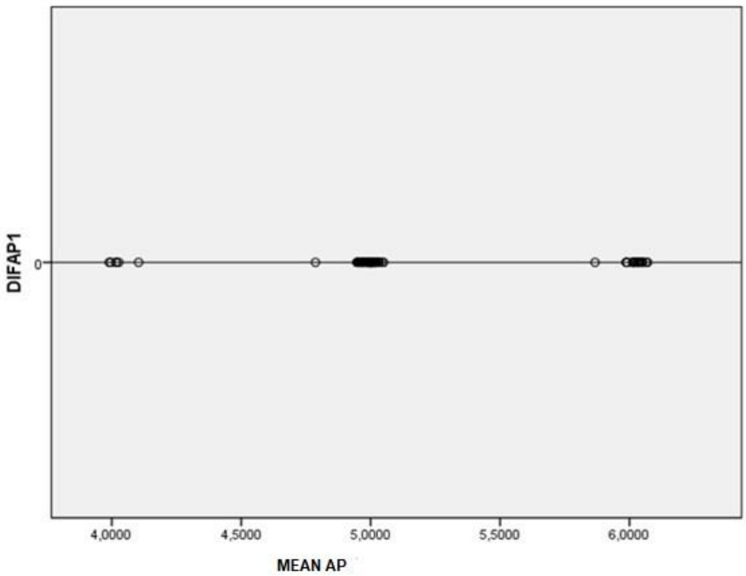

Figure 5: Bland-Altman plots of differences of Phase Angle against mean of Phase Angle.

Simple regression analysis was performed to analyze whether the measurements obtained agreement and whether there is potential bias in the analysis, that is, whether there is a tendency, or whether the values tend to be above or below the mean differences. The value of the mean significance analysis did not show a systematic trend, so there is no proportion bias.

Simple regression analysis was performed to analyze whether the measurements obtained are concordant and whether there is potential bias in the analysis. In other words, if the values tend to be above or below the mean of differences. The value of the mean significance analysis for reactance (p =0.785), resistance (p= 0.715) and phase angle (p>0.7) did not show a systematic trend, so there is no proportion bias

Discussion

Bioelectrical impedance analysis (BIA) is considered a good method for estimating body composition in the epidemiologic studies and at the bedside. It is safe, non-invasive, reliable, rapid, inexpensive, portable, and it allows to repeated measures could be taken quickly [5]. We studied separately R and Xc components grouping by age depending on the sample size and gender. The three age groups adopted were based on Waterloo et al [10] stratification sampling criteria, that clustered the children into relatively homogenous subgroups by age.

In addition, the skin electrodes were placed on anatomical position and those electrodes had their patches width reduced in young children because there is a minimal distance required to avoid interactions between electrodes [16].These criteria adopted by us were similar toother studies in children where: 1) similar groups of children were considered; 2) skin electrodes were placed in accordance with the child's age;3) the children were separated in age–groups; 4) Xc vector component was not neglected; and 5)age-related variability was found in these studies[1,14,15].The measures demonstrated that resistance measurements were substantially higher in all age groups than those reported for adults. In healthy American adults, that means range from432 to 485 ohms for men and 551 to 587 ohms for women and in healthy Brazilian adults 552 +100 ohms in both genders. Our study demonstrated that resistance values in young children were higher than older children, and these results are similar to those in the previous studies [3,5,21]. We observed variability of the resistance and reactance parameters with growth in our study, reinforcing the importance of the reference values of R and Xc by age or age-group and gender in healthy populations of children. The variability of parameters might be reflecting changes during growth as does intra and extra-cellular fluid distribution, cell growth and changes in body mineral and electrolytic content, therefore, reflecting the variability of fluids and body composition in children [22]. The study showed that resistance decreases with age, which might be because the muscular mass of the limbs increases with growth. These observations reinforce the concept whereby in the infants and toddlers, arms and legs represent body area with small diameter and length, therefore the resistance is high. With growth, there is an increase of the diameter and length of the limbs, and R decreases due to an increase in the cross-sectional area of the extremities. These observations are according to simple body-composition models where the appendicular skeletal muscles are the primary electrical conductor [23,24,25,26]. We observed differences in the reactance among the three study-groups. This might be due to the differences of capacitance properties of the tissue interfaces and cell membranes. Theoretically, Xc variation among healthy individuals could be due to differences in the capacitive behavior of the tissues associated with variability of the cell size, membrane permeability or intracellular composition during growth [25,26]. an increase of interstitial fat (anhydrous, meaning that fat is hydrophobic) during maturation reduces both the tissue interface permeability and cell membrane interface permeability, producing an increase in reactance in a critical fixed frequency [27].

The variability of R and Xc might be explained also by variations that include more and less conductive matter, body temperature, tissue composition, fluid distribution, ionic concentration, nature of fat, as well anisotropic effects of muscle fibers. These physiological and structural as well as technical factors affect the measurement of both bioelectrical impedance vector components, R and Xc [3,4,6]. The limitations of this study is that the sample cannot be considered representative of all millions of Brazilian children because there is difference in the nutritional status among specific Brazilian regions depending on the socioeconomic levels of population in each region of Brazil. In order to minimize population bias, the epidemiologic procedure performed in this study consisted of selecting a school with children from families with middle income resources. Our study is the first and only one study already realized in Brazil to establish bioelectrical impedance vectors reference values in children for several age groups and gender.

Conclusion

In conclusion, we established the normative bivariate 90% to 99% confidence intervals for the mean impedance indexes by group and gender and the bivariate predictive values 90% to 99%tolerance limits for new individual measurements of the resistance and reactance in healthy Brazilian children. Further, changes in resistance and reactance with age are well-established. Our findings add substantial information in a field with relative lack of publications.

Contributions

This work was carried out in collaboration among all authors. CMFM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first and final draft of the manuscript. AC Carneluti A managed the literature searches, performed critical analysis and edited the final draft of the manuscript. CMFM and MCA wrote the first draft of manuscript. MCA *performed critical analysis of manuscript and* reviewed the final draft. BIK and WBC *contributed with ideas, methodology and All authors read and approved the final manuscript.*

Consent

As per international standard, parental written consent has been collected and preserved by the author(s).

Ethical

Approval The protocol was approved by the committee of ethics on research of the Universidade Federal de São Paulo and the school's authorities.

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